

Has the Amyloid Cascade Hypothesis for Alzheimer's Disease been Proved?

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Abstract: After much initial debate for and against the role of amyloid in Alzheimer's disease (AD), mutations on the amyloid precursor protein (APP) and processing pathways that increase levels of the amyloid β peptide of 42 residues ($A\beta_{42}$) have established that faulty function or processing of these proteins are responsible for AD pathogenesis. Given the neurotoxicity of aggregates of $A\beta_{42}$, the central role of this peptide in AD pathogenesis is self evident. In this article, I summarize the major pieces of evidence adduced to support the amyloid cascade hypothesis and point out their limitations

INTRODUCTION

For the last 13 years, the amyloid cascade hypothesis has been the dominant organizing principle behind Alzheimer's research [1-3]. This hypothesis has held that the initiating molecule in Alzheimer's disease is $A\beta$. Over these 13 years, the hypothesis have been modified in two significant ways: now the plaques are seen as sinks (and perhaps reservoirs) of toxic $A\beta$ [4] rather than toxic of themselves and the importance of $A\beta_{42}$ as the toxic moiety, rather than total $A\beta$ has become clearer [5].

While many authors have expressed skepticism and dissatisfaction with the amyloid hypothesis, none have suggested any coherent alternative that explains much of the undisputed data concerning disease etiology and pathogenesis.

In this article, I summarize the major pieces of evidence adduced to support the amyloid cascade hypothesis and point out their limitations

1) Mutations in APP which frame the amyloid sequence cause Alzheimer's disease and all these mutations increase the production of $A\beta_{42}$ from APP [6-9].

This evidence proves that something about mutant APP which causes disease. It has been suggested that the fact that it is mutations in APP is just a coincidence and that it is nothing to do with $A\beta$... or that it is loss of some aspect of APP function which initiates disease. Frankly, no coherent alternative to the likelihood that $A\beta$ is the molecule involved in these cases has been suggested. One admitted "problem" with these data is the rarity of APP mutations and the point has been made that it is dangerous to extrapolate from such a small number of cases.

2) Down syndrome individuals get Alzheimer's disease because of over production of APP (and thus $A\beta$) [10].

It used to be argued that Down syndrome cases got Alzheimer's disease because of some other gene rather than overexpression of APP: oxidative stress and chromosome 21 encoded superoxide dismutase was a favourite suggestion: however, cases of Alzheimer's disease who are trisomic distal of APP (and thus triplicated for most genes on the chromosome, including superoxide dismutase) do not develop Alzheimer pathology [11]

3) People with presenilin mutations develop Alzheimer's disease develop Alzheimer's disease because these mutations increase $A\beta_{42}$ [12].

While few now argue that presenilins are directly involved in the cleavage of APP as part of the γ -secretase complex [but see 13], the argument is made that since γ -secretase has other substrates [14], alteration of cleavage of these other substrates, may be important rather than APP: for example, it is clear that presenilin mutations subtly disregulate calcium homeostasis [15] and this has been suggested to be important [16] and the $A\beta$ effect just an epiphenomenon.

4) Transgenic mice with only mutant tau develop tangles in the spinal cord and midbrain [17] but crossing these mice with either APP transgenes [18], or by injection of these mice with $A\beta$ [19] causes tangle formation to occur on other regions.

This experiment has been criticized because, while it clearly shows that APP/ $A\beta$ can influence tangle formation (but not vice versa to a large extent), it is produced in very artificial overexpression system and is not convincing because the "pure" experiment (for example, knock in of a single pathogenic APP mutation) does not cause tangle formation. This criticism is fair, though it should be noted that all transgenic models of neurodegenerative disease require over expression or the engineering of exceptionally pathogenic alleles to cause disease in the lifetime of a mouse.

5) Immunization of mice similar to those described above reduces tau abnormalities [19] proving the link between $A\beta$ and tau [with limited similar data from a few autopsies of those who have been in the Elan immunotherapy trial: ref. 20].

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These experiments can be criticized because tangles are not removed completely by the immunization protocols

6) By analogy: individuals with Worster Drought syndrome: these individuals have mutations which cause amyloid deposition of a completely artificial amyloid (Abri) in a fashion reminiscent of A β in Alzheimer's disease [21]. These individuals also develop tangles and cell loss similar to Alzheimer's disease.

Arguments by analogy can always be criticized.

7) The only accepted risk factor for late onset disease is the apolipoprotein E gene [22]. Mice in which this gene has been knocked out apparently cannot deposit A β [23].

This is admittedly a vague argument and will remain so until the molecular relationship between apolipoprotein and Alzheimer pathogenesis is elucidated.

8) Linkage screen for late onset Alzheimer's disease identify two loci, one on chromosome 21 and one on chromosome 10 whose positions are consistent with proposed loci controlling APP expression or A β production [24-26].

These studies do not constitute hard evidence until the precise molecular basis for them is established.

In each of the eight cases above, there are two alternatives: either one can accept notion that A β is centrally involved in Alzheimer pathogenesis or one can construct a, usually more complex, alternative. However, it has not been possible to construct any alternative that explains a significant proportion of these arguments. It is, of course, possible to adopt a nihilist approach: all cases of Alzheimer's disease don't need to have related causations... but this would seem to be an idea which would lead nowhere and certainly not to therapy

In addition to these criticisms of the positive evidence for the amyloid cascade hypothesis, there are almost mystical suggestions: APP mutations cause "oxidative stress" and this "oxidative stress" (whatever that is) cause amyloid deposition. These suggestions resemble the 19th century discussions of the existence of *phlogiston* (a combustible life force), or the existence of ether to explain the propagation of light in the early 20th century, or the distinction between the mind and the brain in the mid 20th century..... they border on the mystical and untestable, and, as ideas, they eventually wither and disappear from scientific discussion. APP is the precursor of A β (and presenilin is the enzyme responsible for its liberation): surely the simplest way APP (and presenilin) mutations could cause A β deposition is through altering either the amount of A β or its properties?

In addition: there are several misconceptions about the amyloid hypothesis which need addressing. These include the following

1) A β is an important metabolite which is "trying to protect neurons"

While this is a teleological statement, it is certainly possible that A β production is also a part of the response to damage and that it has a neuroprotective function (it would be very interesting to know if tangle formation caused a subtle increase in A β production). Similarly, however, inflammation is a protective and adaptive response but there is no

denying it can be destructive. It is perfectly reasonable to expect that A β has some normal and beneficial function and is certainly very important for us to develop an understanding of APP functions. Similarly, copper is necessary for life but destructive in Wilson's disease: cholesterol is needed, but is important as a risk factor for heart disease.

2) Amyloid deposition does not correlate with the degree of dementia as well as tangle density or synapse loss.

This really is an old chestnut: not only is it fallacious argument since correlation does not prove causation: it also makes the naïve, simplistic and wrong assumption that pathology waits to be counted and, finally, if the prediction is that the amyloid deposition process leads to tangle formation leads to cell loss and circuitry damage leads to dementia, one would expect that correlations would be better between adjacent points in the chain. Thus, rather than being evidence against the amyloid cascade hypothesis, it is a (weak) prediction of it.

3) The complications of the immunotherapy trial are arguments against the amyloid hypothesis.

No they are not: they are complications, which prevented the trial from completely testing the hypothesis.

I am aware of a *schadenfreude* concerning the amyloid-based immunotherapies: a jealous hope that the trials will not be successful. In the face of millions of sick people and their families I regard this guilty pleasure, as irresponsible. But of course, to answer the question posed by the title: the amyloid hypothesis cannot be proved, only disproved: and it will only have been truly useful if it leads to the development of a treatment based on its therapeutic implementation.

The amyloid hypothesis is often ascribed to me or to Dennis Selkoe [1-3]: this is neither true nor fair. We gave expositions of our (similar) views, arrived at independently and brought together bodies of evidence to support those views both 13 years ago and over the intervening period till today. However, Glenner certainly had similar views when he first isolated amyloid- β [27] and one presumes Beyreuther and Masters thought the APP gene was worth the effort of cloning because it was centrally involved in Alzheimer pathogenesis [28]. And the moniker "amyloid cascade hypothesis" was the last published contribution of my erstwhile co-author, Gerry Higgins (1).

STATEMENT

I have no competing financial interests. I observe ethical standards for responsible conduct regarding scientific communication.

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